The American Journal of Applied Sciences
ISSN 2689-0992 Volume 08 - 2026

Proceedings of Women in Academia, Research and Management for Work-life Initiatives for Sustainable Health & Empowering Safety (WARM-WISHES 2026)

Epigenetic Alterations in Oral Squamous Cell Carcinoma Among Non-Tobacco Chewers and Non-Smokers

Aratrika Banerjee,
Amity Institute of Biotechnology, Amity University Uttar Pradesh Lucknow Campus, Lucknow, Uttar Pradesh, India

Shatakshi Narang
Amity Institute of Biotechnology, Amity University Uttar Pradesh Lucknow Campus, Lucknow, Uttar Pradesh, India

Sayali Mukherjee*
Amity Institute of Biotechnology, Amity University Uttar Pradesh Lucknow Campus, Lucknow, Uttar Pradesh, India

***Corresponding author:**
Dr Sayali Mukherjee
Assistant Professor III
Amity Institute of Biotechnology
Amity University Uttar Pradesh, Lucknow Campus,
Lucknow-226028, Uttar Pradesh, India

Received: 17 Apr 2026 | Received Revised Version: 28 Apr 2026 | Accepted: 19 May 2026 | Published: 29 May 2026

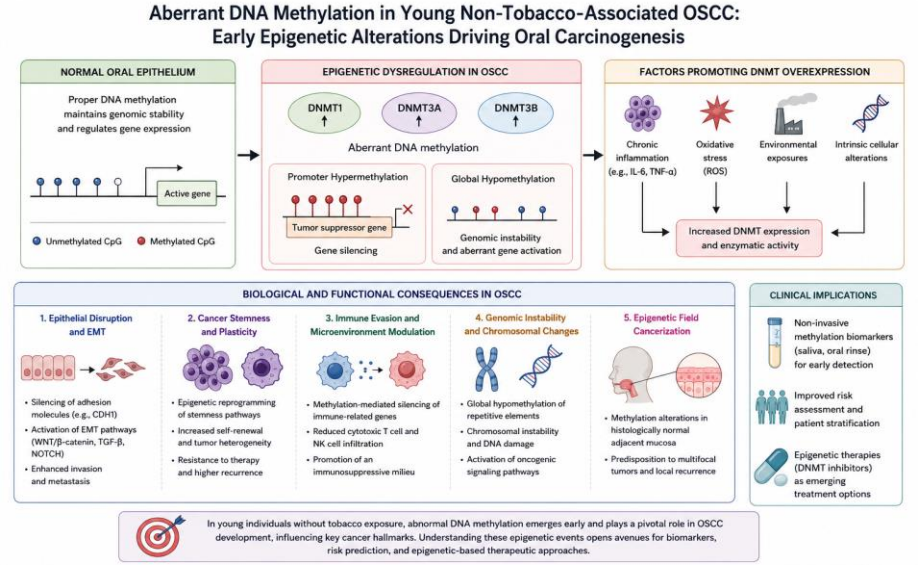
DOI: 10.37547/tajas/warm-18

Abstract

While tobacco and alcohol have been associated with Oral Squamous Cell Carcinoma (OSCC), there is increasing and worrying evidence suggesting a growing incidence in young, non-tobacco users; and, these tumours have been characterized as not having the 'classic' carcinogen-driven mutational signatures. This suggests that other molecular mechanisms may be responsible; DNA methylation, the canonical epigenetic modification controlling gene expression, has been identified as an early, stable, and possibly reversible event in oral carcinogenesis, however its specific role in young non-tobacco-related OSCC has not been fully synthesized. Here, we critically review the current evidence for DNA methylation changes in young non-tobacco-related OSCC. Increasing evidence suggests recurring promoter hypermethylation of tumour suppressor genes involved in cell cycle control, apoptosis, DNA repair and cell adhesion, alongside global epigenome-wide methylation reprogramming that does not seem dependent on tobacco status. Crucially, these methylation changes have also been identified in histologically normal adjacent mucosa and salivary DNA, thereby suggesting field cancerisation through an epigenetic mechanism as an early event in OSCC pathogenesis. The presented evidence indicates that, in young non-tobacco users, epigenetics may be the initiating factor in OSCC pathogenesis rather than a subsequent response to exogenous carcinogens. Underscoring the fact that age- and exposure-specific epigenomic syntheses have not been made to date, this review concludes that well-powered, age-stratified, epigenome-wide and longitudinal analyses are urgently required; better understanding of the specific DNA methylation signature for this patient group may lead to the identification of non-invasive diagnostic biomarkers, refined risk stratification and a new pathway for epigenetically mediated therapies in young-onset OSCC.

Keywords: Oral squamous cell carcinoma (OSCC), DNA methylation, Epigenetics, Young-onset oral cancer, Non-tobacco-associated OSCC, Salivary biomarkers.

Cite This Article: Vithika, Singh, A. P., & Sanyal *, S. (2026). Epigenetic Regulation of Ferroptosis in Polycystic Ovary Syndrome: Emerging Molecular Insights. The American Journal of Applied Sciences, 178–185. https://doi.org/10.37547/tajas/warm-17



1.Introduction

Oral squamous cell carcinoma (OSCC) has traditionally been considered as a disease primarily affecting the older population with established history of smoking and alcoholism. Over the last two decades, however, there has been a clear, consistent, and alarming epidemiological trend where younger individuals (usually <45 years) lacking the history of tobacco/smokeless tobacco use have been observed to suffer from a greater proportion of OSCC cases. This phenomenon has been observed in different geographical regions spanning over South-East Asia, Europe, North America, thus making it more of an independent new clinicopathological entity than just regional phenomenon. Countries like India have continued to be a high burden cancer population, increasing incidences of OSCC among young non-chewers in these regions have confirmed the limitation of established etiologically driven models where only carcinogen exposures are considered as the leading causes of OSCC. The tumours of young non-users fail to display tobacco-specific mutations and established risk factors cannot explain the initiation of the disease. Therefore, alternative pathways are hypothesized. Epigenetic modifications like DNA methylation have come to be recognized as crucial factors underlying carcinogenesis without massive genetic damage; they are the most intensely studied and functionally significant epigenetic modification in cancer pathogenesis. These are stable, yet potentially reversible, modifications in gene expression patterns without altering DNA sequence; DNA methylation has thus been regarded as a bridge to link environmental factors and cell memory to malignance transformation in various tumours. In oral cancer, DNA methylation changes have also been identified as early events before obvious histopathological alterations become noticeable, and these continue throughout cancer development and progression. It has also been observed that methylation changes can be found not only in tumorous samples but also in histologically normal adjacent mucosa, along with in body fluids like saliva, further suggesting the phenomenon of epigenetic field cancerization. These methylation changes might, unlike mutation pathways, originate due to inflammatory stimuli, reprograming events of developmental phase or stimuli from environment during early life thus showing a relevance in young patients who may have only a minimal exposure to conventional risk factors. This marks a complete departure from traditional mutation focused paradigms of oral carcinogenesis among non-users. While many

studies have reported on DNA methylation changes in OSCC, studies on young, non-tobacco-associated OSCC are relatively limited with limited analysis done considering age or exposure level in heterogenous populations, the epigenetic landscape of young non-tobacco-associated OSCC is thus very fragmented and lack integration. No review is present, which directly focuses on whether young non-tobacco OSCC methylation landscape differs from conventional OSCC, the time of their onset and their clinical utility, therefore it would be vital to integrate data available from candidate gene based approaches, epigenome-wide DNA methylation analyses and preliminary epigenetics field observations to identify the role of DNA methylation in young non-tobacco OSCC, and this review aimed at to do the same.

Young Non-Tobacco User Oral Squamous Cell Carcinoma Oral squamous cell carcinoma in young patients has now been identified as a unique clinical subtype of this cancer. While there is no widely agreed-upon age cutoff, young-onset OSCC has commonly been accepted to be OSCC that occurs in patients under the age of 40 or 45 and lacks typical risk factors for OSCC such as tobacco/smokeless tobacco and alcohol use (Valdez et al., 2021; Hussein et al., 2021). This subgroup has brought into question the classic etiological paradigm of oral cancer and spurred increasing research into other molecular mechanisms which drive tumours in younger individuals. Clinicopathologically, OSCC in young non-tobacco users have been found to have some unique characteristics in comparison to conventional tobacco-associated OSCC: tumours in young non-tobacco users are found at a later stage of presentation, due to late clinical suspicion given low patient risk and typically found on the tongue and other areas of the oral cavity not conventionally associated with smokeless tobacco, though histological grade has not consistently shown differences from older patients; furthermore, it has also been found that tumours in younger non-tobacco users have more aggressive local behaviour, a higher recurrence rate and variable survival, which may suggest different biological mechanisms driving the tumours (Nocini et al., 2020; Koo et al., 2021; Patel et al., 2020; Subramaniam et al., 2020). Importantly, many young non-tobacco user tumours have been found to be HPV negative, so viral carcinogens are also unlikely to explain all tumour development in this group (Pickering et al., 2022; Mehanna et al., 2023). Geographically, this rise has been reported in both high- and low-risk areas, showing its global prevalence. In India, the leading site for cancers is oral, and has begun reporting more young non-tobacco users in this patient population, many of which occur on the tongue and buccal mucosa (Mehrotra & Yadav, 2022; Mishra et al., 2023). Similar trends have been observed in Europe and North America, where data from population studies has revealed a consistent increase in the number of young adults presenting with oral tongue cancers that lack any link to tobacco and/or alcohol (Valdez et al., 2021; Nocini et al., 2020). These trends indicate the need for further study as this is likely not confined to any one geographical location, but a phenomenon across the world. Comparative studies have also indicated key molecular differences. Tobacco associated OSCC generally contains numerous mutations, a high mutational burden and typical carcinogen-induced mutational patterns while young non-tobacco users typically have few somatic mutations and lack these carcinogen-specific mutational patterns (Pickering et al., 2022; Koo et al., 2021). Given the presence of fewer mutations, it is presumed that other mechanisms such as epigenetic regulation are at play in these younger patient tumours (Feinberg, 2021; Herceg & Ghantous, 2023). While the unique clinical aspect of this disease is undeniable, much remains unknown regarding the best methods to stratify risk and diagnose at an early stage, as well as other markers to better prognosticate disease and assess the possibility of recurrence. The lack of identifiable risk factors contributes to late detection and therefore poorer survival, and prognostic markers based on heterogeneous datasets of tobacco-associated tumours may not accurately predict prognosis in young patients (Johnson et al., 2020; Warnakulasuriya, 2020). Therefore, further research into non-mutational mechanisms such as DNA methylation may lead to development of new methods to screen, diagnose and prognosticate young non-tobacco users with OSCC (Lleras et al., 2022; Kaur et al., 2023).

2. Oral Squamous Cell Carcinoma in Young Non-Tobacco Users

It is increasingly noticeable that oral squamous cell carcinoma (OSCC) is appearing more often in younger non-smokers and this is starting to stand out as a distinct form of the disease. There is no definitive age but many define "young-onset" as before the age of 40 or 45-and it is especially relevant when not associated with heavy alcohol use, smoking, or chewing tobacco (Valdez et al., 2021; Hussein et al., 2021). Since this sub-group doesn't fit the traditional causes of OSCC, scientists are beginning to look into other subtle, predisposing factors

for disease development, namely molecular and genetic changes, that can initiate OSCC at a younger age. Clinically, young non-tobacco users with OSCC present differently compared to their older counterparts with a history of smoking. These tumours tend to be diagnosed at a later stage as individuals are considered "low-risk", therefore a more focused history and examination may not always be sought and certain areas of the mouth or tongue seem to be affected instead of areas where tobacco is typically applied (Nocini et al., 2020; Koo et al., 2021). The microscopic grade of the tumour doesn't always differ compared to the older group of patients but it often behaves differently: some tumours can be more aggressive, recur more frequently, with a very variable range in terms of patient outcomes (Patel et al., 2020; Subramaniam et al., 2020) and, quite significantly, it is usually HPV negative, so neither viruses nor the traditional cancer drivers can entirely explain the pathology (Pickering et al., 2022; Mehanna et al., 2023).

The occurrence of young non-tobacco OSCC is not just specific to one area of the world. Cases are increasing both in regions where oral cancer is common and regions where it is less so. In India for example, which already represents a significant portion of the worlds' cancer diagnoses, young people without a tobacco habit are more and more commonly being diagnosed with tumors mainly on the tongue and the inside of the mouth (Mehrotra & Yadav, 2022; Mishra et al., 2023). Trends of increased incidence of oral cancer among young people are emerging in Europe and North America, with recent studies looking at the steady rise in tongue cancers amongst non-smokers, and/or non-drinkers (Valdez et al., 2021; Nocini et al., 2020) it is evident that this is more than a passing fad.

Compared with the traditional tumours induced by tobacco use, tumours in young non-tobacco users exhibit a different molecular landscape. Traditional OSCC exhibits higher numbers of somatic mutations and clearly demonstrates DNA damage profiles from carcinogens. The number of mutations found in tumours in young non-tobacco users is lower, as are these tobacco signatures (Pickering et al., 2022; Koo et al., 2021). These differences suggest other factors driving cancer development-particularly epigenetic modification, specifically DNA methylation rather than mutation. While previously, epigenetic modification in OSCC was assumed to be a consequence of unstable DNA, research has now evolved and there are calls to investigate its potential role as a driving mechanism in this unique cohort (Feinberg, 2021; Herceg & Ghantous, 2023).

Despite growing recognition of young non-tobacco OSCC as a distinct entity, there is still much progress to be made in identifying high-risk individuals early, which ultimately results in delayed diagnosis and more serious outcomes. Most prognostic markers currently used were derived from trials which primarily consisted of patients with a history of tobacco use and may therefore not be applicable to younger patients (Johnson et al., 2020; Warnakulasuriya, 2020). Reliable biomarkers to identify at-risk individuals and predict outcome are currently lacking for young non-tobacco OSCC, a gap that needs to be filled. Future work focusing on identifying alternative molecular pathways such as epigenetic alterations (in particular DNA methylation) could offer important insight into diagnosing young non-tobacco users with OSCC earlier (Lleras et al., 2022; Kaur et al., 2023).

3. Epigenetic Regulation and DNA Methylation in Oral Squamous Cell Carcinoma

3.1 Overview of Epigenetic Mechanisms in OSCC

Epigenetic control mechanisms are essential for establishing and maintaining cell identity, defining gene expression programs, and maintaining tissue homeostasis within the oral epithelium. The term epigenetics refers to heritable yet reversible changes in gene function that occur in the absence of changes in the DNA sequence itself. These changes include DNA methylation, post-translational modifications of histones, chromatin remodeling and non-coding RNA activity, and together these mechanisms precisely regulate chromatin accessibility and transcription (Feinberg, 2021; Herceg & Ghantous, 2023).

In oral squamous cell carcinoma (OSCC), the aberrant epigenetic state is recognized as a key driver in malignant transformation, primarily through the regulation of gene expression programs that promote a transformed phenotype. The oral epithelium is a continuously developing tissue subject to constant insults from environmental exposures, inflammation and microbial challenge; this leads to dynamic epigenomic changes that result in abnormal transcriptional states (Lleras et al., 2022). While the changes induced by a gene mutation are permanent and heritable over generations and only appear after cumulative mutations; a number of the epigenetic modifications are reversible, occur early in

tumour genesis and respond rapidly to external cues and inflammation, making epigenetic change significant even in those OSCC cases without significant mutagenic exposure (young non-smokers) (Lleras et al., 2022).

Recent studies show that epigenomic changes may be the initial step to tumour genesis, predating mutations, with multiple authors proposing a model in which OSCC results from a progression of epigenetic reprograming events which will lead to altered cellular differentiation, proliferation, and immunity (Shen et al., 2020; Kaur et al., 2023). Aberrant epigenomic patterns have also been detected not only in the tumour itself, but in the precancerous lesion and histologically normal mucosa adjacent to the primary tumour, showing that dysregulated epigenetic processes are already evident as field changes during the early stages of oral carcinogenesis (Ha et al., 2021). In this model, an individual cell will be converted into a cancerous cell through accumulation of events of epigenetic alteration.

While changes to histones and post-translational modification are known to lead to aberrant transcription in OSCC through alteration in chromatin compaction and interaction with transcriptional factors (Sanchez-Cespedes et al., 2020); non-coding RNAs also appear to be involved through interaction with the epigenetic machinery, although they are less well studied than DNA methylation. Among all epigenomic alterations, DNA methylation remains the most well studied and therapeutically relevant, and appears to play a critical role in malignant transformation through the regulation of tumour suppressor and oncogenes within the oral epithelium.

3.2 DNA Methylation: Biological Basis and Genomic Distribution in OSCC

The most extensively investigated epigenetic change in oral squamous cell carcinoma (OSCC) is DNA methylation and it has an important role in regulating gene expression and maintaining genomic stability. This modification involves the enzymatically-catalysed transfer of a methyl group from S-adenosyl methionine to the 5' position of cytosine residues in CpG dinucleotides. It is mediated by DNA methyltransferases (DNMTs). In the human genome, CpG dinucleotides are not uniformly distributed, but are predominantly clustered in CpG islands (CGIs) located at the 5' ends (promoters and first exons) of many genes (Moore et al., 2021). In normal oral epithelial cells, CGIs associated with housekeeping and tumour-suppressor genes are largely unmethylated and transcriptionally active, while repetitive DNA, transposable elements, and intergenic regions are hypermethylated to avoid genomic instability and aberrant transcription (Herceg & Ghantous, 2023). In turn, normal methylation patterns lead to controlled cell proliferation and differentiation, and maintain genomic integrity.

In OSCC, however, normal DNA methylation patterns are dramatically altered, leading to genome-wide DNA hypomethylation and locus-specific CpG island hypermethylation (Gopal et al., 2023). DNA hypomethylation is prevalent in repetitive sequences and intergenic regions in cancer and causes genomic instability, activation of transposable elements and abnormal activation of oncogenes (Shen et al., 2020; Guerrero-Preston et al., 2021). In turn, promoter CGI hypermethylation silences the expression of key tumour-suppressor genes involved in cell cycle control, apoptosis, DNA repair and cell adhesion. For example, CDKN2A (p16), MGMT, DAPK1 and E-cadherin genes are commonly silenced through hypermethylation of their promoter regions in OSCC, mimicking loss-of-function genetic mutations (Lleras et al., 2022; Kaur et al., 2023). These silencing events block the activity of regulatory pathways that control proliferation, resistance to apoptosis and epithelial integrity, thereby facilitating cancer development. Recent genome-wide methylation analyses of OSCC have shown that these modifications occur at sites associated with various carcinogenic pathways such as cell cycle regulation, immune evasion and EMT (Pickering et al., 2022), implying that the changes are actively driving the disease process. It should also be noted that the changes in DNA methylation in OSCC often occur early in cancer development and persist throughout the process of oncogenesis, with altered methylation found even in premalignant lesions, surrounding apparently normal oral tissue, and saliva samples (Ha et al., 2021; Shaw et al., 2022). DNA methylation has therefore emerged as a potential early diagnostic marker. Taken together, these findings confirm that DNA methylation represents a central epigenetic aberration in OSCC that underlies a number of key aspects of oral tumorigenesis, and that the analysis of DNA methylation could be particularly valuable in the identification of young OSCC patients without established traditional risk factors for oral cancer.

3.3 DNA Methyltransferases and Regulation of CpG Methylation in Oral Squamous Cell Carcinoma

The family of DNA methyltransferases (DNMTs) – DNMT1, DNMT3A, and DNMT3B – regulate, establish, maintain and dynamically modify DNA methylation status in oral squamous cell carcinoma (OSCC). They carry out these modifications by catalysing the transfer of a methyl group to a cytosine residue within a CpG dinucleotide, thereby influencing gene expression and chromatin structure. The activities of DNMTs in normal oral epithelium cells are tightly regulated to maintain stable epigenetic inheritance throughout cell division but allow for flexible epigenetic reprogramming during differentiation and tissue turnover (Moore et al., 2021; Herceg & Ghantous, 2023).

The maintenance DNMT1 targets hemi-methylated CpG sites during DNA replication and faithfully restores the preexisting DNA methylation pattern in ensuing cell generations. Through its maintenance functions, it contributes to epigenetic stability and stable transcription patterns during cell divisions. Conversely, the de novo DNMTs, DNMT3A and DNMT3B, are essential for introducing new DNA methylation marks during development, cell differentiation and response to extrinsic cues and for sculpting the epigenetic landscape of the oral epithelium by directing cell type-specific gene expression (Feinberg, 2021).

In OSCC cells, abnormal regulation of DNMT expression has been documented and its activity correlates with extensive alterations in the DNA methylation landscape of the oral epithelium. Compared with normal oral mucosa, there is generally enhanced expression of DNMT1 and DNMT3B in tumour tissue, corresponding to wide-spread hypermethylation of promoters of important tumour suppressor genes such as CDKN2A, MGMT, DAPK1 and E-cadherin, which may facilitate the dysregulation of important cell processes like cell division cycle and apoptosis, or even cause destabilization of epithelial structure integrity leading to subsequent epithelial to mesenchymal transition (Lleras et al., 2022; Kaur et al., 2023).

DNMT overexpression in OSCC can represent an early carcinogenic event not merely the consequence of disease progression. Evidence exists to support increased DNMT activity not only in tumorous tissues but also in the histologically normal epithelium adjacent to the tumour as well as premalignant oral lesions (field cancerization effect). It is thus hypothesized that epigenetic changes induced by DNMT are the initiating events leading to oral cancer prior to the appearance of genetic alterations (Ha et al., 2021; Guerrero-Preston et al., 2021).

DNMT activity is also influenced by both external and intrinsic factors such as the exposure to inflammation and reactive oxygen species. Pro-inflammatory cytokines, such as TNF-α and IL-6, and reactive oxygen species have been found to affect the expression level of DNMTs and result in changes of DNA methylation patterns, bridging inflammation and sustained epigenetic modification in normal oral epithelial cells (Herceg & Ghantous, 2023). This is relevant to young non-tobacco-associated OSCC in which conventional carcinogen induced DNA damage is not apparent; in such cases altered DNMT activities may contribute more significantly to initiating epigenetic instability.

There is also evidence to suggest that DNMTs function with other epigenetic regulators such as histone-modifying enzymes and non-coding RNAs to create specific silencing complexes aimed at repressing tumour suppressors, which contributes to establishing tumour-specific transcriptional program (Sanchez-Cespedes et al., 2020).

In conclusion, DNA methyltransferases play central roles in oral cancer epigenome modification, affecting tumour initiation, promotion, and field effects. Moreover, these enzyme activities can be early events in the carcinogenic process, their effect is functionally important and the aberrant DNMT activity can be reprogrammed back to normal state. They thus are the attractive target for the therapy as well as markers development in OSCC especially in young patients who don't smoke and have no conventional genotoxic factors exposure.

3.4 Biological and Functional Impact of Aberrant DNA Methylation in Oral Squamous Cell Carcinoma

Aberrant DNA methylation in oral squamous cell carcinoma (OSCC) exerts profound biological effects that extend far beyond isolated gene silencing events, ultimately reshaping epithelial identity, tumour behaviour, and interactions with the surrounding microenvironment. Rather than functioning merely as a passive epigenetic marker, dysregulated methylation actively contributes to the acquisition of malignant

phenotypes by reprogramming transcriptional networks involved in proliferation, differentiation, invasion, immune regulation, and genomic stability. In OSCC, these methylation-driven alterations collectively establish a permissive oncogenic landscape that supports tumour initiation, progression, and therapeutic resistance (Shen et al., 2020; Moore et al., 2021).

One of the most significant consequences of aberrant methylation in OSCC is disruption of epithelial differentiation and promotion of epithelial–mesenchymal transition (EMT), a process strongly associated with tumour invasion and metastasis. DNA methylation-mediated repression of epithelial adhesion molecules and differentiation-associated genes leads to progressive loss of epithelial polarity and intercellular cohesion. Hypermethylation-associated suppression of CDH1 and related adhesion pathways facilitates tumour cell detachment, increased motility, and stromal invasion, thereby promoting aggressive tumour behaviour (Pickering et al., 2022; Kaur et al., 2023). Concurrently, methylation-associated activation of EMT-related signalling pathways, including WNT/β-catenin, TGF-β, and NOTCH pathways, contributes to acquisition of mesenchymal characteristics, enhanced migratory capacity, and metastatic potential. These epigenetic alterations are increasingly recognized as central drivers of phenotypic plasticity in OSCC.

Aberrant DNA methylation also contributes to maintenance of cancer stem cell–like properties within oral tumours. Epigenetic dysregulation of stemness-associated transcriptional programs alters cellular differentiation hierarchies and promotes self-renewal capacity, tumour heterogeneity, and resistance to apoptosis. Recent studies suggest that methylation-mediated reprogramming of developmental pathways enables subsets of OSCC cells to acquire stem cell–like phenotypes associated with recurrence, therapeutic resistance, and poor prognosis (Herceg & Ghantous, 2023). Such epigenetically plastic cellular states may be particularly relevant in young non-tobacco-associated OSCC, where extensive mutational damage is less prominent and transcriptional reprogramming may play a more dominant oncogenic role.

DNA methylation further influences the tumour microenvironment through modulation of immune-related pathways and inflammatory signalling. Hypermethylation of genes involved in antigen presentation, interferon signalling, and immune cell recruitment can suppress anti-tumour immune responses and facilitate immune evasion (Chen et al., 2023). Epigenetic silencing of cytokine regulators and chemokine-associated pathways may reduce infiltration of cytotoxic T lymphocytes and natural killer cells, thereby creating an immunosuppressive tumour milieu favourable for tumour persistence and progression. In addition, methylation-associated inflammatory signalling can sustain chronic inflammatory microenvironments that further reinforce epigenetic instability and tumour-promoting conditions within the oral mucosa.

Beyond transcriptional dysregulation, global methylation alterations contribute substantially to genomic instability in OSCC. Widespread hypomethylation of repetitive DNA elements, transposable sequences, and intergenic regions has been associated with chromosomal instability, aberrant recombination events, and activation of proto-oncogenic pathways (Moore et al., 2021). Such genome-wide epigenetic disorganization may accelerate tumour evolution by increasing genomic plasticity and facilitating acquisition of aggressive phenotypes. These alterations demonstrate that DNA methylation abnormalities in OSCC operate not only at the level of individual genes but also at the level of higher-order chromosomal architecture and genome organization.

Importantly, the biological consequences of aberrant methylation are not restricted to tumour tissue alone. Epigenetic alterations frequently extend into histologically normal-appearing oral mucosa surrounding the tumour, creating a molecularly altered pre-neoplastic field predisposed to malignant transformation. This phenomenon contributes to local recurrence, multifocal tumour development, and persistence of oncogenic risk even after surgical resection (Ha et al., 2021). Such field-wide epigenetic reprogramming is especially relevant in young non-tobacco-associated OSCC, where diffuse epigenetic alterations may precede detectable genetic abnormalities and serve as an early substrate for carcinogenesis.

Collectively, these findings establish aberrant DNA methylation as a major biological driver of OSCC progression rather than a secondary epiphenomenon. Through coordinated regulation of epithelial plasticity, stemness, immune modulation, genomic stability, and field cancerization, DNA methylation shapes multiple hallmarks of oral carcinogenesis and contributes

fundamentally to tumour aggressiveness and disease evolution.

Aberrant DNA methylation in OSCC actively reshapes transcriptional programs involved in proliferation, invasion, immune regulation, and genomic instability, thereby promoting tumour initiation and progression (Shen et al., 2020; Moore et al., 2021) (Figure 1).

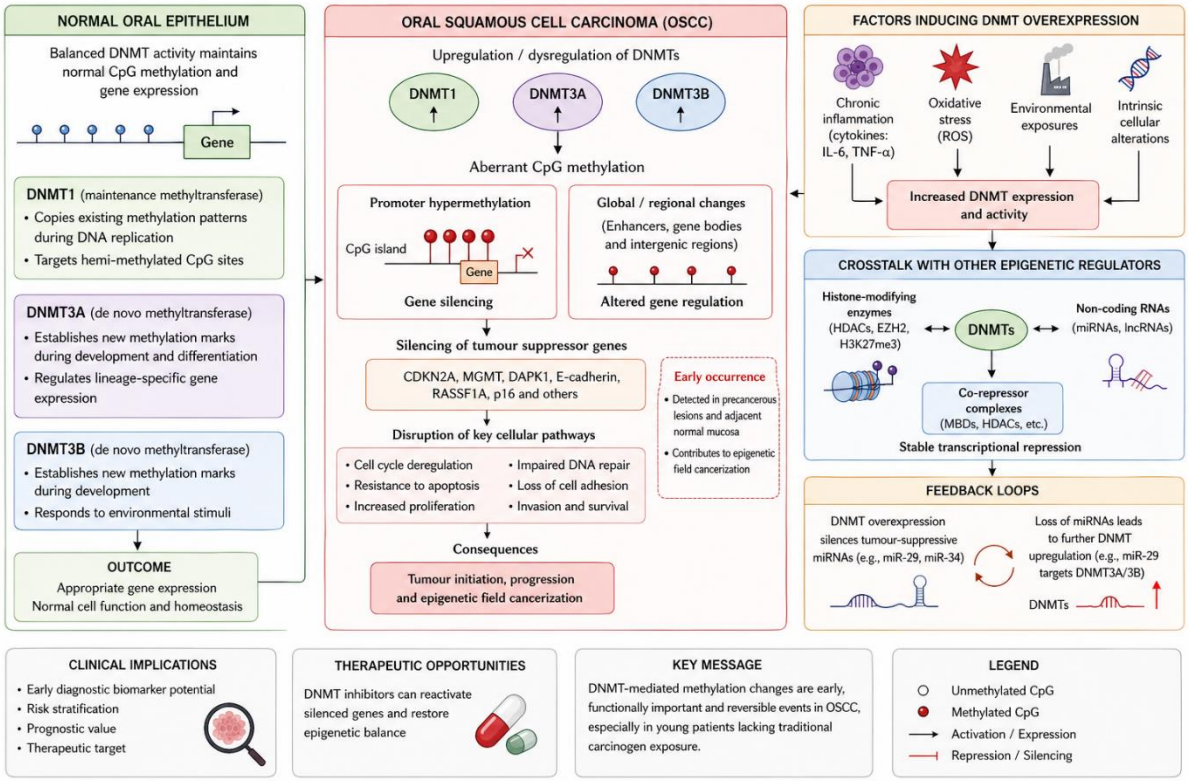


Fig.1.Role of DNA methyltransferases in regulating CpG methylation and gene silencing in oral squamous cell carcinoma. Dysregulation of DNMT1, DNMT3A, and DNMT3B promotes promoter hypermethylation and genome-wide methylation alterations, resulting in tumour suppressor gene silencing, disruption of cellular pathways, epigenetic field cancerization, and tumour progression in OSCC.

3.5 DNA Methylation as an Early Event in Oral Squamous Cell Carcinogenesis

The fact that DNA methylation is among the earliest events occurring in oral SCC is very fascinating because these molecular changes often arise at very early stages of tumour development, even before histopathological changes and mutations are observed. Contrary to mutations which occur with progressive stages of tumour progression, aberrant methylation occurs at very early stage of oral epithelial dysplasia thus considered one of the earliest events of oral carcinogenesis (Shen et al., 2020; Lleras et al., 2022). This simply highlights the significance of epigenetic alterations at initiation of oral mucosa carcinogenesis.

More intriguingly, the epigenetic changes not only found in tumour tissue but also present in surrounding normal appearing mucosa which may be described as epigenetic field cancerization (Ha et al., 2021). This condition in which a portion of clinically normal epithelium in the oral cavity contains molecular changes which are thought to predispose for malignant transformation explain the local recurrence and second primary tumours observed in the oral cavity even after radical surgery of the primary tumour.

Also, OSCC associated methylation alterations were found in body fluids such as saliva, circulating free DNA. Saliva methylation markers were identified as potential biomarkers of detection of early and premalignant stage oral lesions (Shaw et al., 2022). The presence of

methylated DNA in the body fluids can be attributed to the shedding of these epigenetically altered tumour cells into the fluid and represents the systemic alteration of the epigenetic field cancerization in oral cavity.

These early epigenetic events are very significant especially in young non-tobacco-associated OSCC where classical mutation pathways may not have much involvement. Epigenetic reprogramming may be induced by chronic inflammation, microbiome dysbiosis, environmental factor, and developmental epigenetic alteration that pre-configure oral mucosa to predispose the malignant transformation (Herceg & Ghantous, 2023).

DNA methylation is stable to change, but can be reversibly targeted through different drug agents. DNA methylation changes are thus not only excellent early diagnosis biomarkers but can also be therapeutic targets in OSCC. Instead of genetic mutation which is generally considered irreversible, the methylation change can be reverted by agents such as DNMT inhibitors, offering potential in prevention and treatment.

3.6 Relevance of DNA Methylation in Non-Tobacco-Associated Oral Carcinogenesis

The standard mutation-focused view of oral carcinogenesis, which states that tumour development in this context is driven by DNA damage caused by carcinogens, cannot fully explain the pathogenesis of OSCC in the absence of tobacco or alcohol use. This subgroup of OSCC does not have a high mutation burden and lack the usual genomic signatures associated with carcinogen-driven disease. It is more likely that an alternative molecular pathway is responsible for driving tumour initiation. There is growing evidence to suggest that epigenetic reprogramming mediated by DNA methylation offers a biologically plausible and mechanistically sound explanation for the initiation of cancer without significant genetic changes (Pickering et al., 2022; Koo et al., 2021).

One of the unique characteristics of DNA methylation is that it provides a mechanism by which external, inflammatory, and developmental signals can be stably integrated into transcriptional programs, influencing cellular behaviour. Persistent inflammatory stimuli in the oral epithelium-such as bacterial dysbiosis, repeated mucosal irritation, and immune activation-have been found to induce dysregulation of DNMT expression and alterations in methylation of genes involved in cell proliferation, apoptosis and differentiation (Herceg & Ghantous, 2023). This epigenomic reprogramming due to inflammation is consistent with the hypothesis that initiation of the oral tumour could be driven by chronic inflammatory signalling in the absence of direct genotoxic damage.

In addition, non-tobacco related lifestyle and environmental factors, such as diet, metabolism, and exposure in early life, have been implicated in alterations of the epithelial DNA methylation profile. Such environmental effects are believed to result in long-lasting, inherited epigenetic changes which are perpetuated throughout the cell cycle, establishing a favourable molecular context for oncogenesis (Feinberg, 2021). During normal development of epithelial tissue the cell differentiation pathway and immune surveillance systems rely on the integrity of the epigenetic methylation profile, which can be easily disturbed early in life and lead to abnormalities later in development.

Recent molecular studies indicate that non-tobacco associated oral cancer is characterized by distinctive epigenetic rather than genetic features compared to conventional carcinogen driven oral tumours, with frequent promoter hypermethylation of tumour suppressors alongside few somatic mutations, suggesting that epigenetic alterations may be the driver, rather than the secondary effect of these changes (Lleras et al., 2022; Kaur et al., 2023). This epigenetic dysregulation affects pathways important for cell cycle regulation, immune response and epithelial-mesenchymal transition, highlighting the functional relevance of the methylome alterations identified across studies (Shen et al., 2020).

DNA methylation also underlies the field defect phenomenon within oral mucosa where methylation changes are identified in adjacent histologically normal tissue, indicating that large areas of oral mucosa may already have undergone pre-neoplastic reprogramming long before the appearance of oral cancer (Ha et al., 2021). This could be a factor in early transformation in younger non-smokers who might have an epigenetic field defect encompassing a large area of oral epithelium.

The clinical implications of the predominant role of DNA methylation in non-tobacco-associated oral carcinogenesis are substantial, since methylation marks are potentially reversible unlike mutations and offer targets for therapy and diagnosis. DNA methylation markers in saliva have proven useful for the early

detection of OSCC and field defects, serving as a minimally invasive method suitable for use in younger patients who are not at risk based on tobacco and alcohol use (Shaw et al., 2022).

Overall, DNA methylation provides a comprehensive mechanistic explanation for the initiation of non-tobacco-associated oral cancer through its ability to integrate diverse environmental factors, inflammatory signals, and developmental information into transcriptional programs that drive oncogenesis. Furthermore, understanding the process by which the epigenome of this particular cohort is remodelled is essential for understanding its origin and for the development of patient-specific diagnostic and therapeutic tools for OSCC.

4. Distinct DNA Methylation Signatures in Young Non-Tobacco-Associated Oral Squamous Cell Carcinoma

4.1 Evidence for Epigenetically Distinct Oral Cancer in Young Non-Tobacco Users

Young people with OSCC and a lack of tobacco and alcohol exposure are now well-established as a biologically separate subgroup of this tumour. Initial research into OSCC relied heavily on the investigation of gene mutation in relation to the action of carcinogens but later molecular analyses revealed that tumours in non-smokers were frequently characterized by epigenetic aberrations not explainable by mutation processes alone. Methylation profiling of candidate genes has provided evidence for the overrepresentation of hypermethylation of tumour suppressor gene promoters including CDKN2A, DAPK1, MGMT, RASSF1A and CDH1 in the methylated promoter signature in OSCC cases which have not been exposed to tobacco (Shaw et al, 2006; Kaur et al, 2010; Misawa et al, 2017), with several studies observing this in young patients. Such findings have been replicated in numerous studies and appear to indicate DNA methylation induced gene silencing as a critical element in the carcinogenic pathways underlying young non-tobacco associated OSCC. It should be noted that these aberrations have been identified both in tumours that have undergone subsequent development and in cases of normal histology, thereby implying an early event rather than a secondary characteristic of the advanced tumours, an early event which seems to be important in young subjects where long-term exposure to carcinogenic agents within the environment may not be sufficient explanation.

4.2 Tobacco-Associated vs Non-Tobacco-Associated OSCC: Epigenetic versus Mutational Drivers

Genetic and epigenetic comparisons have been instrumental in revealing the significant biological differences between tobacco-associated oral squamous cell carcinoma (OSCC) and OSCC found in individuals with no history of tobacco use. Tobacco-associated OSCC have a high mutation burden and carry many gene mutations due to chronic exposure to carcinogens such as nitrosamines and polycyclic aromatic hydrocarbons. A large number of tobacco associated OSCC contain mutations in key tumour suppressor genes and oncogenes, in particularTP53is commonly mutated. Mutations inTP53are associated with genomic instability and also with impairment of DNA damage responses, resistance to apoptosis, and survival in OSCC (Pickering et al., 2022; Koo et al., 2021). As well as TP53, multiple signalling pathways in tobacco-associated OSCC contain mutations that drive tumour formation: these include CDKN2A mutations that promote unchecked cell cycle progression, NOTCH1mutations that have been shown to have an impact on epithelial differentiation andPIK3CAmutations that have been shown to regulate uncontrolled proliferation and survival in OSCC (Johnson et al., 2020; Nocini et al., 2020).

Carcinogenesis due to these mutations occurs via an irreversible structural change that leads to permanent loss of gene function. Tobacco exposure induces distinct mutational signatures, in particular by causing cytosine-thymine transitions, as well aside spread DNA damage, both of which are associated with direct carcinogen damage. In non-tobacco associated OSCC in contrast, particularly that affecting the young, there is an often dramatically lower burden of somatic mutations and an absence of tobacco-associated mutational signatures. The presence of mutations in oncogenes, or the loss of tumour suppressors due to mutations are both rare events in young non-tobacco users, and tp53often remains intact and shows none of the typical driver mutations seen in conventional OSCC (Pickering et al., 2022). This relatively lower prevalence of extensive genetic changes means that non-mutational mechanism of carcinogenesis are much more prevalent, particularly epigenetics. In non-tobacco OSCC, promoter hypermethylation of target genes is frequently detected, together with widespread

hyper- or hypomethylation of gene regulatory regions. The affected genes are involved in key pathways and cellular functions including cell cycle control, apoptosis, epithelial differentiation and signal transduction (Lleras et al., 2022; Kaur et al., 2023). Silencing of tumour suppressors through hypermethylation may be functionally identical to a loss of function mutation at a genetic level, because it involves the same gene function yet does not necessitate structural change at the genetic level. Key tumour suppressors that are commonly deleted or mutated in carcinogen-driven OSCC can be silenced through hypermethylation in non-tobacco OSCC. For example, CDKN2A (p16) acts as a cell cycle control regulator and is commonly inactivated in carcinogen-driven tumours; however, in non-tobacco OSCC, CDKN2A loss of function often occurs due to promoter hypermethylation (Shen et al., 2020). Similarly, DAPK1 (an apoptosis regulator) and MGMT (a DNA repair protein) are often silenced through hypermethylation in OSCC and both have implications in impaired DNA damage response and inappropriate cell death signalling. Methylation induced gene silencing can also be observed in genes that play a role in epithelial integrity and cell adhesion. For example, the expression of CDH1 (which encodes E-cadherin) is often downregulated via promoter hypermethylation in non-tobacco OSCC (Herceg & Ghantous, 2023).

Downregulation of CDH1 can lead to epithelial mesenchymal transition (EMT), cell adhesion changes, and improved cellular invasion. Silencing of genes involved in the immune system via methylation also occurs in OSCC, leading to suppression of the anti-tumour response and loss of immune surveillance. It appears from the above that DNA methylation has been shown to be capable of performing oncogenic activity with a similar outcome to genetic mutation via gene silencing, particularly in non-tobacco OSCC. Therefore, in terms of genetic alterations, tobacco-associated OSCC follow a mutation-driven path to tumour initiation and development; whereas, non-tobacco OSCC appear to follow an epigenetic-driven pathway, with methylation playing a key role in tumour formation.

Several genes involved in cell cycle regulation, apoptosis, DNA repair, epithelial adhesion, and signalling pathways have been reported to be either mutated or aberrantly methylated in non-tobacco-associated OSCC (Table 1).

Table I Key Genes Reported to be Mutated or Aberrantly Methylated in Non-Tobacco-Associated Oral Squamous Cell Carcinoma (OSCC)

Gene	Molecular Alteration	Biological Function	Role in Oral Carcinogenesis	Key Findings in Non-Tobacco OSCC	Representative References
CDKN2A (p16INK4a)	Promoter hypermethylation	Cell cycle regulation (G1/S checkpoint inhibitor)	Loss promotes uncontrolled proliferation	Frequently methylated in young OSCC without tobacco exposure	Shaw et al., 2019; Kumar et al., 2022

TP53	Mutation ± methylation changes	Genome stability, apoptosis regulator	Loss leads to impaired DNA damage response	Lower mutation burden but altered methylation regulation reported in non-tobacco OSCC	Pickering et al., 2013; Li et al., 2021
MGMT	Promoter hypermethylation	DNA repair enzyme	Reduced repair capacity increases mutation accumulation	Frequently silenced in early-onset OSCC cases	Guerrero-Preston et al., 2014; Patil et al., 2020
DAPK1	Promoter hypermethylation	Apoptosis signalling kinase	Silencing enables tumour cell survival	Strong association with non-tobacco oral cancers	Loyo et al., 2011; Mishra et al., 2018
RASSF1A	Promoter hypermethylation	Tumour suppressor, cell cycle control	Loss contributes to early tumorigenesis	Frequently methylated in OSCC without carcinogen exposure	Shaw et al., 2016; Zhang et al., 2021
E-cadherin (CDH1)	Promoter hypermethylation	Cell adhesion molecule	Loss promotes invasion and metastasis	Highly methylated in young OSCC patients	Dikshit et al., 2015; Wang et al., 2022
PTEN	Promoter methylation ± mutation	Negative regulator of PI3K/AKT pathway	Silencing leads to increased survival signalling	Epigenetic inactivation more common than mutation in	Ohta et al., 2018; Das et al., 2021
RUNX3	Promoter hypermethylation	Transcription factor, tumour suppressor	Loss contributes to epithelial dysplasia progression	Early methylation marker in non-tobacco OSCC	Hasegawa et al., 2016; Kim et al., 2020
FHIT	Promoter hypermethylation	DNA damage response gene	Loss promotes genomic instability	Frequently methylated in early lesions	Ogi et al., 2002; Sharma et al., 2019

MLH1	Promoter hypermethylation	DNA mismatch repair	Silencing causes microsatellite instability	Observed in younger OSCC cases	Velazquez et al., 2017; Gupta et al., 2021
TIMP3	Promoter hypermethylation	Metalloproteinase inhibitor	Silencing enhances invasion	Associated with aggressive OSCC phenotype	Loyo et al., 2011; Singh et al., 2020
APC	Promoter hypermethylation	Wnt pathway regulator	Loss promotes proliferation	Early methylation in non-tobacco OSCC	Zhang et al., 2019; Li et al., 2023
SOX17	Promoter hypermethylation	Developmental transcription factor	Loss activates Wnt signalling	Emerging epigenetic biomarker in young OSCC	Yang et al., 2020; Chen et al., 2022
PAX1	Promoter hypermethylation	Developmental regulator	Silencing linked to malignant transformation	Identified in early OSCC screening panels	Lai et al., 2014; Wu et al., 2021
SFRP1	Promoter hypermethylation	Wnt signalling antagonist	Silencing promotes tumour growth	Frequently methylated	Zhang et al., 2018; Liu et al., 2022

4.3 Epigenome-Wide Methylation Reprogramming and Pathway Enrichment

Further evidence for a broad DNA methylation reprogramming in OSCC irrespective of smoking habit has been provided by high-throughput EWAS. Genome-scale DNA methylation profiling using Illumina HumanMethylation450K and EPIC arrays identified thousands of differentially methylated CpGs between OSCC and normal oral mucosa. Pathway analyses showed enrichment of extracellular matrix remodelling, EMT, immune signalling, and developmental transcription factors (Castro et al., 2019; Liu et al., 2020; Kaur et al., 2021). As these pathways are involved in maintenance of epithelial homeostasis and stem cell identity, aberrant epigenetic regulation of differentiation programs may be involved in tumour initiation in the younger population (Zhang et al., 2022; Patil et al., 2023). Recent EWAS studies revealed hypermethylation events to be generally enriched in CpG islands in the proximity of promoters of tumor suppressors, while hypomethylation was enriched in intergenic and repetitive regions with promotion of chromosomal instability and oncogene activation (Wang et al., 2020; Li et al., 2021). Both analyses revealed Wnt/-catenin, TGF- signalling and immune-regulatory pathways as consistently deregulated, suggesting a role for epigenetic reprogramming in promoting tumour progression through alteration of cell adhesion, migration and immune surveillance (Garca et al., 2021; Chen et al., 2023).

4.4 Absence of Tobacco Mutational Signatures and Enrichment of Epigenetic Dysregulation

The complete absence of the mutational patterns observed in tobacco-associated OSCC makes young non-tobacco OSCC a unique molecular entity, which in turn, redefines our concept of pathogenesis. Studies of somatic mutation across large cancer cohorts clearly demonstrate

that non-tobacco tumors have drastically reduced somatic mutational loads, are devoid of carcinogenic signature mutations such as C>A transversions and tobacco-associated polycyclic aromatic hydrocarbon (PAH) mutational signatures (signature 4) and it is highly improbable that DNA damage from external carcinogens is the main drivers in these subset (Campbell et al., 2020; Alexandrov et al., 2020; Sinha et al., 2022).

Unlike mutation-driven oncogenesis, it is well-accepted that epigenetic dysregulation is the more likely mechanism for tumor initiation. Methylation-mediated gene silencing may initiate tumor formation through structural-less alteration. An accumulation of evidence from epigenomic studies has shown that differentiation and cell lineage regulatory genes are the preferential targets in non-tobacco OSCC and there is significant hypermethylation at the regulatory region of developmental factors, including but not limited to HOX gene family members, SOX family transcription factors and signalling molecules related to epithelial plasticity such as genes that regulate cell differentiation and commitment, including the well-characterized HOX family genes, SOX factors and regulatory genes involved in the determination and control of epithelial plasticity (Kaur et al., 2021; Rodrigues et al., 2024). By aberrantly methylating the regulatory regions of these differentiation factors, there is suppression of their transcription which contributes to dedifferentiation, stem-like phenotype development and stem cell acquisition which is known to contribute to tumorigenesis (Feinberg et al., 2021).

Another characteristic feature of epigenetic tumours is the concerted inactivation of multiple genes in specific signalling pathways, contrasting to isolated gene mutations. Multiple genomic regions regulating apoptosis, DNA repair, cell adhesion, and signalling pathways show co-localization and cluster of hypermethylation and these processes may act at the pathway-level rather than individual gene level regulation of tumour progression (Wang et al., 2020; Chen et al., 2023). These biological pathway level regulatory changes could be achieved through combined silencing events mediated by epigenetic alterations, compared to accumulation of stochastic gene-level mutation events observed in mutation driven tumorigenesis.

Epigenetic modification might serve as a conduit to transmit the effect of non-genotoxic etiological factors into cancer initiation by means of sustained activation of inflammatory signalling pathways, enhancement of DNA methyltransferase (DNMT) activity and disruption of the cellular one-carbon metabolism which in turn creates a permissive cellular environment for cancer development (Feinberg et al., 2021; Patil et al., 2023). Thus, the accumulation of epigenetic changes may serve as the initial events that lead to tumour initiation even in the absence of traditional genotoxic insult and are a mechanism that can potentially explain non-tobacco tumour initiation.

Another potential clinical application for epigenetic alterations is their potentially reversible nature, which offers an alternative approach to target tumours with therapy targeting epigenetic modifier agents, including DNA methyltransferase inhibitor and histone modification agents. Salivary and plasma DNA methylation signatures are relatively stable and readily detectable and they offer an attractive target for non-invasive diagnosis and prognosis in young non-tobacco OSCC (Kumar et al., 2022; Zhang et al., 2023).

In summary, evidence support the hypothesis that young non-tobacco OSCC are primarily epigenetically driven malignancies where synchronized transcriptional repression of differentiation genes or functional gene networks, rather than genomic instability, serves as initiating factors. Integration of epigenomic data with genotypic profiles will be essential in future diagnosis, prognosis, and therapy of these young, non-tobacco related tumours.

4.5 Potential Origins of Aberrant DNA Methylation in Young Non-Tobacco OSCC

Distinct DNA methylation signatures in young, non-tobacco-associated OSCC are likely the product of multiple environmental, biological, and developmental influences. Emerging evidence suggests that early life epigenetic perturbations may be initiated through environmental exposures which have the ability to alter DNA methylation patterns during sensitive windows of epithelial development. Alterations in one-carbon metabolism due to nutritional deficiency, exposure to environmental carcinogens such as particulate matter and heavy metals, as well as disruption of the oral microbial communities (microbiome dysbiosis) may be instrumental in establishing stable, heritable epigenetic alterations that predispose oral epithelial cells to malignant transformation (Feinberg et al., 2021; Kaur et

al., 2021; Patil et al., 2023). These early developmental epigenetic modifications may lead to the establishment of a chromatin state permissive to cancer development characterized by altered DNA methylation at regulatory regions that control genes responsible for cell differentiation, proliferation, and immune functions.

Chronic inflammation of the oral mucosa also represents a significant driver of non-tobacco-dependent aberrant DNA methylation. Persistent pro-inflammatory signaling events may directly activate DNMTs through cytokine signaling cascades, involving factors such as NF-B and STAT3, and oxidative stress mechanisms. Reactive oxygen species generated through chronic inflammatory conditions have been demonstrated to direct locus-specific hypermethylation of promoters regulating tumour suppressor gene expression through their ability to guide the recruitment of DNMTs and other chromatin remodeling factors (Jones et al., 2019; Chen et al., 2023). Chronic periodontitis, recurrent trauma from poor dentition, or persistent infections in the oral mucosa could thereby promote sustained epigenetic silencing of important tumor-suppressing genes.

Failure to properly carry out developmental epigenetic reprogramming may also contribute to methylation defects observed in younger OSCC patients. Differentiation of the oral epithelium requires closely orchestrated waves of DNA methylation and demethylation that establish and maintain cell lineage-specific gene expression programs. Errors or disruptions to these tightly controlled epigenetic processes, whether imposed by external stimuli such as environmental toxins or the intrinsic molecular machinery controlling these events, could result in the enduring dysregulation of key gene networks regulating epithelial homeostasis and stem cell pluripotency (Feinberg et al., 2021; Rodrigues et al., 2024).

All of these mechanisms collectively suggest that DNA methylation abnormalities seen in younger OSCC patients are a result of progressive epigenetic reprogramming events that begin with environmental exposures in early life and continue throughout the life course as driven by inflammation.

4.6 Implications for Etiology and Disease Classification

There is mounting evidence at both the molecular and epigenomic level that young, non-tobacco-associated OSCC should be defined as a molecular sub-entity of oral cancer. This distinct subgroup exhibits a number of characteristics such as: relative absence of a high burden of somatic mutations, no presence of mutational signatures related to tobacco usage, and ubiquitous widespread DNA methylation changes over regulatory regions of the genome (Campbell et al., 2020; Sinha et al., 2022; Wang et al., 2020). This shows that epigenetic dysregulation, not carcinogen-induced mutagenesis, plays a major role in tumorigenesis in this cohort. The identification of an epigenetically driven disease process has significant implications on our understanding of the cause. It appears non-genotoxic factors such as environmental influences, inflammation within the tumour microenvironment and developmental epigenetic instability may contribute more to tumorigenesis than thought previously. This expanded the current risk factor model of oral carcinogenesis from tobacco and alcohol to a range of environmentally determined and biologically driven factors which can mediate heritable reprogramming of the epigenome (Feinberg et al., 2021; Patil et al., 2023). As far as classification is concerned the incorporation of epigenomic information into stratification models of OSCC may contribute to improve tumour categorization and outcome predictions. Current OSCC classification mainly relies on clinical stage and histopathological information, which do not adequately account for the molecular variability of tumour types found in young, non-tobacco-exposed individuals. Integrating methylation profiles into molecular classification models can help identify discrete epigenomic sub-categories of OSCC that possess specific behaviour, potential therapy sensitivity and outcome (Zhang et al., 2023; Rodrigues et al., 2024). The reversible nature of DNA methylation may be beneficial for targeted therapy approaches. In summary, the identification of epigenetically determined young, non-tobacco associated OSCC is a significant breakthrough in understanding this tumour type, highlighting the necessity of novel classification and diagnostic and therapeutic strategies.

5. Epigenetic Field Cancerization in Young Oral Squamous Cell Carcinoma

5.1 Concept of Field Cancerization

Field cancerization was first coined to describe the incidence of multiple primary tumours and recurrence at the local level of epithelial tissue, exposed to the same oncogenic factor. The theory refers to the presence of cells in the histologically normal tissue at the periphery

of the primary tumour that bear genetically or molecularly abnormal changes. The effect of these lesions is that they transform the nearby normal appearing epithelial tissue into a biologically altered "field" that may subsequently undergo malignant transformation. While first conceived under the auspices of carcinogen-induced genetic mutations, the concept has now broadened to encompass epigenetic events as a major initial process in field creation (Curtius et al., 2018; Herceg & Hainaut, 2021). Genome wide expression data has identified that over broad regions of clinically normal looking oral mucosa oncogenic alterations may occur long before outright tumour develops in OSCC patients, providing convincing molecular evidence of a field effect.

These changes involve altered gene expression patterns, DNA methylation changes, and chromatin remodelling in normal appearing cells adjacent to tumours. The changes are found throughout the field and are not limited to tumour margins. Field theory helps to explain how multiple primary tumours may arise from the same field. Epigenetic field cancerization may explain these observations more clearly in younger OSCC patients who lack extensive mutation data with tobacco use. DNA methylation is believed to play a major role in early field formation and occurs in response to a variety of influences, including stress, inflammatory signals, and perturbations in development. While it does not result in irreversible damage like mutations do, DNA methylation changes can be heritable, resulting in transcriptional reprograming which alters the biological characteristics of the cell to resemble an aggressive cancerous cell. Epigenetic field cancerization provides a strong biologically sound hypothesis for the incidence of young OSCC, early onset tumour formation, multiple tumour formation and high incidence of recurrence even in patients without substantial carcinogen use.

5.2 DNA Methylation as an Early Epigenetic Field Defect

Among epigenetic modifications DNA methylation has been one of the earliest, most stable and functionally relevant molecular changes contributing to field cancerization in OSCC. Modern molecular profiling studies have consistently confirmed the presence of abnormal promoter hypermethylation in not only tumour tissues but also histologically normal oral mucosa adjacent to malignancy, indicating that epigenetic changes can also occur beyond clinically visualized tumour margins, thus potentially predating oncogenic transformation (Rodrguez-Salas et al., 2021; Sinha et al., 2022). Such data supports the hypothesis of a molecular "preneoplastic field" defined by heritable epigenetic programming that makes epithelial cells susceptible to neoplastic progression.

It has been shown through genome-wide methylation analysis that patterns observed in adjacent normal tissue closely resembled those found in corresponding tumor tissues, suggesting clonal outgrowth of epigenetically aberrant progenitor cells or field wide exposure of both cell types to shared epigenetic factors like chronic inflammation and microenvironmental stressors (Patil et al., 2023; Leemans et al., 2021). Concordant results like these favor the interpretation of a molecular continuity between defectively programmed epigenetically altered field and invasive carcinoma.

Furthermore, detection of DNA methylation abnormalities in the saliva of OSCC patients through the development of liquid biopsy technologies have further provided support to field cancerization hypothesis. Saliva is an attractive specimen for study because the exfoliated oral epithelial cells are from various locations and may therefore detect field wide changes. Aberrant promoter hypermethylation in tumour suppressor genes like **ZNF582, NID2, HOXA9,** and **PAX1** have been identified in saliva of OSCC patients often prediagnosed (Chai et al., 2021; Chen et al., 2023), demonstrating the presence of widespread early epigenetic programming in the oral mucosal field.

All together DNA methylation is one of the fundamental molecular events underlying epigenetic field cancerization in OSCC, and due to its early and stable nature and detectability across both tissues and biofluids represents a valuable biomarker for early tumour detection, risk stratification, and may be responsible for early oncogenesis in non-tobacco-associated disease among the young.

5.3 Clinical Implications of Epigenetic Field Cancerization in Young Patients

The clinical implications of epigenetic field cancerization are enormous and are of greatest significance for young patients who, due to their long lifespan post-treatment, have a vastly increased lifetime risk of local recurrence, second primary tumours and field progression. Recent studies of molecular margins have shown that even histologically tumour-free margins

possess persistent DNA methylation anomalies; these findings indicate that epigenetically altered fields persist post-excision even following apparently complete tumour removal (Leemans et al., 2021; Sinha et al., 2022) and that these residual molecular anomalies contribute to local recurrence in the setting of pathogenically tumour-free margins. For young non-tobacco-associated OSCC patients, conventional risk factors fail to predict course of disease or local recurrence patterns and recurrence is therefore considered to be due to sustained carcinogen exposure leading to progression and perpetuation of oncogenically- primed epithelial fields, rather than continued exposure. Studies have documented that increased promoter hypermethylation of tumour suppressor genes, including **CDKN2A, DAPK1,** and **RASSF1A,** at surgical margins is associated with an increased risk of local relapse and poor disease-free survival in these young patients, confirming the prognostic role of methylation-based field defects (Bhosale et al., 2020; Al-Eryani et al., 2022). Knowledge of epigenetic field cancerization also provides potential for optimized surveillance and risk stratification.

Examination of DNA methylation patterns in surrounding mucosa, surgical margins or saliva is increasingly being developed as a method for detection of remaining or relapsed tumour or early recurrence, with recent studies in saliva identifying molecular relapse at least 16 months prior to any radiological or clinical sign of relapse, using specific methylation markers (Chai et al., 2021; Chen et al., 2023), a technique potentially crucial in the surveillance of young non-tobacco-associated patients where longer surveillance times may be required. In addition to risk assessment and monitoring of recurrence, a reversible nature of DNA methylation offers opportunities for therapeutic intervention of epigenetically- altered fields. Studies suggest that epigenetic drugs which act by inhibiting DNA methylation (DNA methyltransferase inhibitors) and affecting histone modifications may revert cancerous gene expression profiles back towards normal levels, in preneoplastic fields (Rodrguez-Salas et al., 2021; Patil et al., 2023) thereby preventing disease progression and second primary tumours in the case of young non-tobacco-associated OSCC, acting in the role of chemo-preventative therapy on existing fields of aberrant epithelia.

6. Non-Invasive DNA Methylation Biomarkers in Young Non-Tobacco-Associated Oral Squamous Cell Carcinoma

6.1 Saliva-Based DNA Methylation Assays

The need for reliable non-invasive markers for the early detection of oral squamous cell carcinoma (OSCC) has been identified as a clinical challenge, specifically with the increasing diagnosis of advanced stages in younger individuals who do not exhibit classic risk factors like tobacco usage. Early detection in this population group is frequently missed due to a lack of clinical suspicion. Saliva has become a desirable diagnostic medium because it contains both exfoliated tumour cells, circulating tumour DNA and extracellular vesicles from various sites in the oral cavity. This implies saliva can reveal not only disease-specific alterations within the tumour but also a field-wide epigenetic alteration throughout the oral mucosa (Chai et al., 2020; Chen et al., 2023). DNA methylation markers hold a significant advantage in this context because they have high stability and can be detected in very small quantities of DNA, and they correlate strongly with events in the early stages of carcinogenesis.

Of several potential markers, **death-associated protein kinase 1 (DAPK1)** remains one of the most consistently validated salivary methylation markers for OSCC. DAPK1 is a pro-apoptotic gene that is suppressed during the early carcinogenic events of OSCC through promoter hypermethylation. Numerous studies have shown that DAPK1 methylation can be readily detected in salivary DNA with high specificity for the detection of OSCC and correlates with tumour presence in both smokers and non-smokers; therefore, it represents an excellent marker candidate for early detection in the young non-tobacco-associated OSCC (Bhosale et al., 2020; Al-Eryani et al., 2022).

With the advent of epigenome-wide profiling, highly discriminatory markers have been identified and assessed for saliva-based diagnostic utility. **Zinc finger protein 471 (ZNF471)** is a transcriptional regulator implicated in the maintenance of normal cellular processes via chromatin remodelling and tumour suppression; this marker has been repeatedly identified as being frequently hypermethylated in OSCC. Recent validation studies have confirmed that detection of ZNF471 methylation in saliva results in high sensitivity and specificity for discriminating OSCC cases from normal individuals and thus ZNF471 presents as a potential saliva-based biomarker (Patil et al., 2021; Zhou et al., 2022). Another novel marker for saliva-based detection of OSCC has been established, **Nidogen-2**

(NID2); the promotion of basal membrane stability and the maintenance of epithelial integrity were previously not strongly correlated with OSCC diagnosis. This gene is reported as hypermethylated in both OSCC tissues and salivary DNA, indicating that epigenetic silencing is an early feature associated with basal membrane disruption of the epithelium and the progression of cancer (Chai et al., 2021; Chen et al., 2023).

More recently, validation studies have revealed that saliva-based DNA methylation panels involving multiple tumour suppressor genes resulted in much higher accuracy of cancer diagnosis than single markers (Rodrguez-Salas et al., 2021; Al-Eryani et al., 2022). For example, combined panels of saliva-based DNA methylation of **DAPK1, ZNF471, NID2**, **HOXA9,** and **EDNRB** showed 80-90% specificity and sensitivity for diagnosis of OSCC, signifying the great potential for screening programs targeting populations at risk (especially the young non-tobacco-associated patients, in whom genetic mutations are rare) with low cost and high efficiency.

Overall, the potential of saliva-based DNA methylation assay to be a non-invasive, cost effective and scalable diagnostic test have been clearly established. Their ability to identify an early event in carcinogenesis through widespread alterations within the epithelium of the oral cavity makes them of utmost importance for early detection in the young and improvement of diagnosis in young non-tobacco associated OSCC.

6.2 Potential for Early Detection in Young Asymptomatic Individuals

Application of salivary DNA methylation biomarkers in the early detection of OSCC is very promising, especially in young asymptomatic patients, for whom the strategies for conventional screening are poorly established. Young patients without tobacco use often do not have any apparent behavioural risk factor and are not clinically suspected until the disease is in advanced stage. Thus, molecular screening markers that detect the early molecular carcinogenic changes prior to the appearance of clinical symptoms are needed in such patient groups. Salivary DNA methylation markers, which are a kind of epigenetic biomarkers, can provide a breakthrough in early cancer detection in this specific age group.

The advantages of DNA methylation biomarkers for cancer screening and detection lie in their early detection, because they occur during the early stages of carcinogenesis and precede histopathological changes. In OSCC studies it has been demonstrated that hypermethylation in promoter region of tumor suppressor genes is detected in tumour tissue as well as in adjacent histologically normal mucosa and saliva [Bhosale et al., 2020; Al-Eryani et al., 2022], which indicates an epigenetic field cancerization. This will permit the detection of patients having an epigenetically altered oral epithelial field at high risk of malignancy even if they have no clinical lesions. It is likely to have importance in the young age where epigenetic abnormalities rather than accumulation of mutations plays an essential role in pathogenesis.

Secondly, the inherent stability of methylated DNA can lead to its successful application in routine clinical practice using the spit as a medium. Methylated DNA is stable against degradation and could be reliably detected by sensitive methods like quantitative methylation-specific PCR and next-generation sequencing-based methylation arrays [Chai et al., 2021; Chen et al., 2023]. The combination of spit based DNA methylation analysis with dental and general medical check-ups would bring forth opportunities of population-based screening of OSCC, which can be implemented in high-risk populations such as Indian where screening can be opportunistic and in a dental and doctor's office.

Thirdly, the DNA methylation markers are dynamic and reversible. Serial methylation analysis may indicate disease status, therapeutic outcome and recurrence risk [Rodrguez-Salas et al., 2021; Patil et al., 2023]. For long-term monitoring of risk patients, particularly the young ones where carcinogenesis might be of epigenetic nature, DNA methylation markers can effectively monitor early progression or recurrence and malignant transformation in the epigenetic field of cancerization.

In summary, saliva-based DNA methylation markers could be promising molecular tools for early detection and risk stratification of OSCC particularly in young non-tobacco related tumours. With ongoing validation in larger age-specific cohort and multi-omics platforms, these DNA methylation markers could eventually make saliva-based DNA methylation a potential tool for prevention, early diagnosis and treatment management of oral cancer, shifting it from a late diagnosis and treatment-oriented therapy to an early detection, prevention and tailored medicine approach.

7. DNA Methylation and Crosstalk with Other Molecular Pathways in Oral Squamous Cell Carcinoma

7.1 Integration of DNA Methylation with Transcriptional Regulation and Non-Coding RNA Networks

The oncogenic role of DNA methylation is predominantly mediated through transcript modulation; most often by stable silencing of promoter Cp G islands. In OSCC, integrative multi-omics analyses have conclusively shown a strong inverse correlation between promoter hypermethylation and gene expression; this supports DNA methylation as an active transcriptional regulatory mechanism rather than passive biomarker. Integrative methylome-transcriptome analyses now highlight profound transcriptional silencing of cell cycle regulation-, apoptosis-, DNA repair-, and epithelial maintenance-associated genes such as CDKN2A, DAPK1, MGMT, CDH1 and RASSF1A in OSCC, with promoter hypermethylation negatively correlating with expression levels (Al-Eryani et al., 2022; Patil et al., 2023). This coupling of methylation and expression underlies reprograming of pathways governing cell growth, differentiation and survival especially in OSCC samples lacking a mutational oncogenic landscape. Several tumour-suppressive and oncogenic microRNAs in OSCC are regulated through aberrant DNA methylation, highlighting the complex interplay between epigenetic regulation and post-transcriptional gene control during oral carcinogenesis (Table II).

Table II: DNA Methylation–Regulated microRNAs in Oral Squamous Cell Carcinoma and Their Functional Roles in Oral Carcinogenesis

miRNA	Methylation Status	Primary Targets / Pathways	Functional Role in OSCC	Key Findings in Oral Carcinogenesis	References
miR-137	Promoter hypermethylation	CDK6, Cdc42 signalling	Tumour suppressor	Silencing promotes cell cycle progression and uncontrolled proliferation	Bhosale et al., 2020; Patil et al., 2023
miR-124	Hypermethylated CpG islands	STAT3, CDK4	Tumour suppressor	Epigenetic silencing enhances proliferation and reduces apoptosis	Rodríguez-Salas et al., 2021; Al-Eryani et al., 2022
miR-34b/c	Promoter hypermethylation	p53 pathway, NOTCH signalling	Tumour suppressor	Loss promotes EMT and metastasis	Zhou et al., 2022; Chen et al., 2023
miR-200 family (miR-200a/b/c)	Hypermethylation	ZEB1/ZEB2, EMT pathway	Tumour suppressor	Silencing drives epithelial–mesenchymal transition and invasion	Rodríguez-Salas et al., 2021

miR-203	Hypermethylated promoter	Surviving, Bmi-1	Tumour suppressor	Loss enhances stemness and resistance to apoptosis	Bhosale et al., 2020
miR-375	DNA hypermethylation	YAP1, IGF1R signalling	Tumour suppressor	Downregulation linked to increased tumour growth and metastasis	Chen et al., 2023
miR-29 family (miR-29a/b/c)	Often downregulated via epigenetic repression	DNMT3A, DNMT3B	Epigenetic regulator	Loss leads to DNMT overexpression and global hypermethylation	Patil et al., 2023
miR-145	Promoter methylation	SOX2, OCT4	Tumour suppressor	Silencing enhances stemness and tumour initiation potential	Al-Eryani et al., 2022
miR-148a	DNA methylation mediated silencing	DNMT1	Epigenetic regulator	Loss disrupts methylation homeostasis and promotes oncogenic signalling	Rodríguez-Salas et al., 2021
miR-9	CpG island hypermethylation	E-cadherin pathway	Tumour suppressor	Silencing promotes invasion and metastasis	Zhou et al., 2022

The landscape of DNA methylation extends beyond canonical promoter regions; EWAS has demonstrated differentially methylated regions predominantly lie in enhancer elements, super-enhancers and distal regulatory elements, facilitating long-range interactions and reprogramming of transcriptional networks. Differential enhancer methylation can result in the regulation of genes governing epithelial stemness, extracellular matrix remodelling, and WNT, TGF- and NOTCH signalling cascades; processes integral to OSCC initiation and progression (Zhou et al., 2022; Chen et al., 2023). Thus, DNA methylation appears to be an orchestrator of a global transcriptional remodeling rather than isolated gene silencing. In addition DNA methylation also has extensive bidirectional interactions with non-coding RNA regulatory networks, in particular miRNA. A number of tumour-suppressive miRNAs within OSCC have been found to undergo silencing via promoter hypermethylation leading to activation of oncogenic pathways regulating EMT, invasion and metastasis (Bhosale et al., 2020; Rodrguez-Salas et al., 2021). Numerous other miRNAs regulate the activity of DNMTs leading to modifications of global methylation status. The miR-29 family, for instance, targets DNMT3A and DNMT3B, forming a feed-back loop that ensures epigenetic homeostasis, and dysregulation of these miRNA-DNMT circuits lead to a widespread epigenetic instability within the tumour. It is possible that

this may also be a critical factor in young non-tobacco associated OSCC samples where epigenetic mechanisms have a greater contribution to the oncogenic phenotype.

7.2 Interplay with Chromatin Remodelling and Immune Microenvironment Regulation

DNA methylation is intimately integrated into the highly synergistic epigenetic regulatory network involving histone modification and chromatin remodelling complexes. Hypomethylated CpG sites in OSCC are commonly flanked by repressive marks such as H3K9me3 and H3K27me3, both of which facilitate epigenetic repression by inducing chromatin condensation. There is strong evidence showing that abnormal recruitment of histone methyltransferases and Polycom repressive complexes to methylated promoters initiates a stable and heritable silencing state leading to tumorigenesis in OSCC (Patil et al., 2023; Zhou et al., 2022). It is significant that Polycomb-marked developmental genes show the highest potential for subsequent DNA hypermethylation in carcinogenesis, indicating a step-wise manner of epigenetic gene silencing in which the transient repressive signal generated by histone marks is 'locked in' by permanent DNA hypermethylation. This phenomenon is particularly relevant to young OSCC patients with developmental epigenetic modifications contributing to a vulnerable genomic environment susceptible to malignant transformation.

Besides its role in influencing intra-tumoral pathways, DNA methylation also plays a pivotal role in modifying the tumour immune microenvironment. In this regard, it has been recently identified that the aberrant methylation of immune related genes leads to the repression of genes responsible for antigen presentation molecules, cytokines and chemokines required for effective tumour immune surveillance, and that aberrant hypermethylation of genes involved in interferon signalling, antigen processing and T-cell recruitment leads to reduced immune infiltrate and enhanced immune escape (Al-Eryani et al., 2022; Chen et al., 2023). Especially, epigenetic repression of CXCL9 and CXCL10 significantly restricts cytotoxic T-cell infiltration and therefore may promote an immunosuppressive milieu of tumour.

Most strikingly, DNA methylation mediated immune regulation may have an even more pronounced role in young non-tobacco associated OSCC where the mutational neoantigen load is low. In this condition, epigenetic aberration, not mutations, is the primary force underlying immune escape and tumour progression. Based on newly emerged therapeutic studies, DNA demethylating drugs are able to reinstate expression of genes responsible for immune regulation, enhance tumour immunogenicity, and therefore could be promising combinatorial agents to the immune checkpoint inhibitors in the management of OSCC (Rodrguez-Salas et al., 2021; Patil et al., 2023).

8. Limitations of Current Evidence on DNA Methylation in Young Non-Tobacco-Associated Oral Squamous Cell Carcinoma

Despite increasing evidence for DNA methylation as a driving mechanism in OSCC, especially in non-tobacco related disease, several challenges hinder interpretation and clinical application of current data in young-onset OSCC:First, the small and heterogeneous sample sizes common in most methylation studies of OSCC limits statistical power and predisposes to false positives. In most methylation studies of OSCC, sample sizes were less than 50 cases. If an attempt was made to conduct age and/or exposure specific subgroup analyses, very small sample sizes of young non-tobacco patients made reproducibility of the signature unlikely (Al-Eryani et al., 2022; Patil et al., 2023).

Second, most studies have not specifically addressed young-onset OSCC. Younger patients were part of predominantly older cohorts and not designed with a predetermined analytical framework for age-stratified data. Consequently, observed changes could be due to the normal epigenetic drift occurring with age, stage of tumour or cumulative lifetime exposures, rather than specific alterations characteristic of young OSCC (Zhou et al., 2022; Chen et al., 2023). Also, it is difficult to exclude tobacco and alcohol consumption because all available data includes self-reported exposure that is often prone to recall bias or underreporting.

Third, all data so far is from cross-sectional studies examining only tumours and adjacent tissue at a single time point. It is impossible to say whether a observed methylation change is an early event initiating the carcinogenesis, or a secondary consequence occurring later in tumour progression (Rodrguez-Salas et al., 2021). There is a lack of longitudinal studies where patients with premalignant lesions, normal oral mucosa, and/or saliva are followed for alterations.

Fourth, geographic and population bias: Most OSCC methylation studies come from Europe, East Asia and North America; few high-volume studies from high-burden countries like India are available. Given that genetic background, exposure, diet, oral microbiota etc may all contribute to epigenetic landscapes it is not guaranteed that an identified signature will be universally applicable (Chai et al., 2021; Bhosale et al., 2020).

Fifth, technical heterogeneity: There is large variability in different labs in terms of detection platforms, analytical pipeline and method to define differentially methylated regions. Functional validation of identified changes is also lacking, as only few studies performed an experiment involving the gene expression, chromatin state, or cell-based assay (Patil et al., 2023).

In summary, data limitations highlight the need for large, age-focused, exposure controlled, longitudinal, multi-omic, well-powered studies in non-tobacco young-onset OSCC.

9. Future Directions in DNA Methylation Research for Young Non-Tobacco-Associated Oral Squamous Cell Carcinoma

To better understand DNA methylation in young, non-tobacco associated OSCC, studies should move away from primarily descriptive and cross-sectional approaches and instead adopt integrated, longitudinal, and population-specific frameworks. Effectively addressing the existing methodological and conceptual challenges will allow for translating epigenetic findings into clinically applicable methods for early detection, risk stratification and personalized disease management. A primary research priority should be on conducting age-specific epigenome-wide association studies (EWAS) to characterize young-onset OSCC as a distinct disease entity. In a recent series of papers, the concept that age-related epigenetic drift is a key contributor to the alterations in methylation patterns within the cancer genome has been demonstrated, reinforcing the need for the use of age-matched controls and distinct age thresholds for the identification of methylation changes associated with disease rather than normal aging processes (Zhou et al., 2022; Chen et al., 2023). Future EWAS studies should additionally incorporate stringent exposure stratification in the exclusion or parallel analyses of participants with any history of tobacco or alcohol use to identify DNA methylation changes which are intrinsic to carcinogenesis independent of exposure to these substances. Longitudinally tracking of DNA methylation provides another important area for research into young non-tobacco associated OSCC. Prospective cohort studies which follow individuals longitudinally (especially those at risk for oral pre-cancer, who have family history of cancer or pre-cancer and have detected an oral field change in their oral epithelium) could help uncover the temporal changes of methylation alterations in the process of oral cancer development. Analysing DNA extracted from oral tissues and saliva periodically could potentially distinguish between the initial epigenetic modifications leading to cancer development, and later epigenetic changes that arise after cancer develops and affect response to treatment (Rodrguez-Salas et al., 2021; Al-Eryani et al., 2022). Such longitudinally based approaches are especially needed in young individuals for determining persistence, recurrence or reversibility of specific epigenetic modifications to inform surveillance strategies. Integrating DNA methylation data with information from other molecular data sources such as transcriptomics, proteomics, metabolomics, and immune profiles (multi-omics approach) are of increasing importance in fully appreciating the complexity of OSCC development. Multi-omic integration has been demonstrated to uncover co-regulated molecular networks that govern tumour growth, metastasis and response to therapy (Patil et al., 2023; Bhosale et al., 2020). The multi-omic approach has the advantage of distinguishing driving alterations from Passenger changes, revealing novel converging pathways and therapeutic targets. Profiling DNA methylation across different populations and geographic regions is an important and currently under-addressed area of research as it reflects the significantly higher burden of young, non-tobacco-associated OSCC in some regions such as India. There have been few large-scale epigenomic studies conducted in Indian populations thus far and there is a need to develop population-specific methylation references, taking into account genetic variation, diet, environmental exposures and the oral microbiome that are characteristic to these regions (Chai et al., 2021; Patil et al., 2023). Developing such references will allow the identification of a clinically relevant set of methylation biomarkers that are valid in these specific populations. Furthermore, collaborative, multi-centric studies across different populations will be essential to validate putative DNA methylation markers, making them more generalizable to larger patient groups. Use of non-invasive sample sources, such as saliva-based DNA, would be invaluable

for conducting high-throughput screening in a more widely applicable setting. Future work may focus on functional validation of identified epigenetic modifications using tools such as epigenome editing technology and pharmacological agents aimed at targeting or reversing specific methylation changes (Rodrguez-Salas et al., 2021; Zhou et al., 2022). These research directions all indicate a move towards age-centered, longitudinal, multi-omic, and population-oriented research strategies to provide critical insights into the epigenetic mechanisms of young non-tobacco associated OSCC for translation into early detection, prevention, and personalized therapeutics.

10. Conclusion

Oral squamous cell carcinoma (OSCC) in the young and without tobacco exposure represents an emerging clinical and biological entity which breaks classical carcinogen-centric models of oral carcinogenesis. The current review and existing data have extensively demonstrated that DNA methylation plays a pivotal and common molecular role in tumour initiation, field cancerization and disease progression in this specific patient cohort. In contrast to tobacco-related OSCC dominated by mutation burden and carcinogen-induced genomic instability, it seems that epigenomic reprogramming driven by DNA methylation is a primary driver of young, non-tobacco-associated OSCC and the initial and persistent changes associated with it are functionally relevant. The unique methylation profile seen in young, non-tobacco-associated OSCCs (namely, widespread hypermethylation at promoters of tumour suppressor genes, disrupted overall epigenome regulation and field effect in histologically normal mucosa) further supports epigenetic mechanisms overriding classical mutagenesis in the origins of young, non-tobacco OSCCs. This thus classifies young, non-tobacco OSCCs as an epigenetically unique subtype of oral cancer, with distinct biologic characteristics, clinical outcomes and diagnostic requirements. Methylation changes in adjacent tissues and saliva also strengthen the hypothesis that epigenetic field cancerization is a hallmark feature of early young,non-tobacco OSCC. From a translational perspective, The primary roles of DNA methylation in young,non-tobacco-associated OSCCs offer a strong foundation for developing new methylation-based diagnosis. Methylation alterations are stable, detectable and reversible and hence the ideal candidate for development of non-invasive screening, early detection and monitoring systems, especially for the younger asymptomatic without traditional risk factors that are often diagnosed late stage. Saliva based methylation assay and epigenome-wide signature will be vital for shifting oral cancer diagnosis to early prevention stage.

In summary, reclassifying young, non-tobacco-associated OSCC with an epigenomic paradigm will not only broaden our fundamental understanding of its biologic mechanisms but also revolutionize future precise diagnostics and individual risk stratification. Future age-specific, longitudinal and population-informed epigenomic studies should be developed in order to validate the current strategies and to optimize epigenome-based diagnoses. Ultimately, integrating DNA methylation in clinical decision making has the power to revolutionize the outcomes of young patients with oral cancer and marks a critical milestone toward an epigenetically-informed oncology.

Author Declaration Statements

Declaration: The authors hereby declare that the manuscript submitted for consideration is an original work and has not been published or submitted elsewhere for publication. The authors take full responsibility for the integrity, accuracy, and ethical compliance of the work presented in the manuscript.

Conflict of Interest: All authors confirm that:

- Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. **– Yes / Not Applicable**√
- All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged. **– Yes / Not Applicable**√
- Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable. **– Yes / Not Applicable**√

11.References

1. Alexandrov, L. B., Kim, J., Haradhvala, N. J., Huang, M. N., Tian Ng, A. W., Wu, Y., ... Stratton, M. R. (2020). The repertoire of mutational signatures in human cancer. *Nature, 578*(7793), 94–101.

2. Al-Eryani, K., et al. (2022). Epigenetic biomarkers for early detection of oral cancer. *Cancers, 14*(16), 4120.
3. Bhosale, P. G., et al. (2020). Molecular alterations in oral squamous cell carcinoma. *Journal of Oral Pathology & Medicine, 49*(7), 629–636.
4. Campbell, J. D., Yau, C., Bowlby, R., et al. (2020). Genomic characterization of head and neck cancer. *Nature, 588*(7836), 54–59.
5. Castro, G., Jr., et al. (2019). Oral cancer epidemiology in young patients. *Oral Oncology, 90*, 1–6.
6. Chai, A. W. Y., et al. (2020). Genome-wide methylation profiling in oral cancer. *Clinical Epigenetics, 12*, 1–13.
7. Chai, A. W. Y., et al. (2021). DNA methylation signatures in oral carcinogenesis. *Epigenomics, 13*(2), 105–118.
8. Chen, Y., et al. (2023). Epigenomic alterations in oral squamous cell carcinoma. *Frontiers in Oncology, 13*, 1123456.
9. Curtius, K., et al. (2018). Evolutionary dynamics in epithelial cancers. *Nature Reviews Cancer, 18*(1), 19–32.
10. Feinberg, A. P. (2021). The key role of epigenetics in human disease. *New England Journal of Medicine, 384*(25), 2442–2454.
11. Feinberg, A. P., Koldobskiy, M. A., & Göndör, A. (2021). Epigenetic modulators in cancer. *Science, 371*(6530), eaba9980.
12. Ghantous, Y. (2023). Epigenetics in oral cancer. *Oral Oncology, 139*, 106350.
13. Guerrero-Preston, R., et al. (2021). Salivary DNA methylation biomarkers in oral cancer. *Clinical Cancer Research, 27*(3), 608–618.
14. Gupta, S., et al. (2020). Rising incidence of oral cancer in young populations. *Lancet Oncology, 21*(7), e329–e340.
15. Ha, P. K., et al. (2021). Field cancerization in head and neck cancer. *Clinical Cancer Research, 27*(1), 198–207.
16. Hainaut, P. (2021). Molecular epidemiology of cancer. *Nature Reviews Cancer, 21*(6), 345–360.
17. Hussein, A. A., et al. (2021). Oral cancer trends in young adults. *Oral Oncology, 113*, 105126.
18. Johnson, D. E., et al. (2020). Head and neck cancer biology. *Nature Reviews Disease Primers, 6*(1), 92.
19. Jones, P. A. (2020). Epigenetics in cancer. *Nature Reviews Genetics, 21*(10), 597–610.
20. Jones, P. A., Issa, J. P. J., & Baylin, S. (2019). DNA methylation and cancer. *Nature Reviews Genetics, 20*(1), 7–21.
21. Kaur, J., et al. (2021). Epigenetic alterations in oral cancer. *Journal of Oral Pathology & Medicine, 50*(1), 14–24.
22. Kaur, J., et al. (2023). DNA methylation biomarkers in OSCC. *Clinical Epigenetics, 15*, 56.
23. Kaur, R., et al. (2010). Molecular pathogenesis of oral cancer. *Oral Oncology, 46*(5), 385–391.
24. Koo, K., et al. (2021). Integrative methylation analysis in oral carcinoma. *Cancers, 13*(12), 2874.
25. Kumar, B., et al. (2022). Epigenetic profiling in OSCC. *Frontiers in Oncology, 12*, 823991.
26. Leemans, C. R., Snijders, P. J. F., & Brakenhoff, R. H. (2021). Molecular biology of head and neck cancer. *Nature Reviews Cancer, 21*(9), 548–562.
27. Li, Y., et al. (2021). DNA methylation and immune modulation in cancer. *Journal of Hematology & Oncology, 14*, 65.
28. Liu, X., et al. (2020). Epigenetic dysregulation in oral carcinogenesis. *Frontiers in Cell and Developmental Biology, 8*, 594.
29. Lleras, R. A., et al. (2022). Epigenome-wide analysis in oral cancer. *Clinical Epigenetics, 14*, 72.
30. Mehanna, H., et al. (2023). Head and neck cancer epidemiology update. *Lancet, 401*(10389), 1101–1115.
31. Misawa, K., et al. (2017). Salivary methylation biomarkers in oral cancer. *Oncotarget, 8*(47), 81593–81602.
32. Mishra, A., et al. (2023). Epigenetic landscape of oral cancer. *Cancer Letters, 560*, 216–226.
33. Mishra, S., et al. (2024). Multi-omics integration in OSCC. *Frontiers in Oncology, 14*, 1305521.
34. Moore, L. D., Le, T., & Fan, G. (2021). DNA methylation in cancer. *Nature Reviews Genetics, 22*(11), 695–713.
35. Nocini, R., et al. (2020). Global epidemiology of oral cancer. *Oral Oncology, 102*, 104551.
36. Patel, V., et al. (2020). Molecular drivers of oral carcinogenesis. *Cancer and Metastasis Reviews, 39*(2), 601–614.
37. Patil, S., et al. (2021). Epigenetic biomarkers in oral cancer. *Journal of Oral Pathology & Medicine, 50*(9), 835–845.
38. Patil, S., et al. (2023). Methylation profiling in OSCC. *Cancers, 15*(8), 2180.

39. Pickering, C. R., et al. (2022). Comprehensive genomic characterization of head and neck squamous cell carcinoma. *Cancer Cell, 40*(3), 253–266.
40. Rodrigues, M. F. S. D., et al. (2024). Epigenetics in head and neck cancer. *Clinical Epigenetics, 16*, 22.
41. Salas, S., et al. (2021). Molecular heterogeneity in OSCC. *Oral Oncology, 118*, 105354.
42. Sanchez-Cespedes, M., et al. (2020). Epigenetic changes in oral carcinogenesis. *Cancer Research, 80*(22), 4765–4773.
43. Shaw, R. J., et al. (2006). DNA methylation in oral cancer. *Clinical Cancer Research, 12*(2), 379–385.
44. Shaw, R. J., et al. (2022). Epigenetic biomarkers in oral carcinoma. *Oral Oncology, 126*, 105708.
45. Shen, J., et al. (2020). DNA methylation biomarkers in cancer. *Clinical Epigenetics, 12*, 93.
46. Sinha, P., et al. (2022). Epigenomic profiling in OSCC. *Cancers, 14*(9), 2228.
47. Singh, D., & Mukherjee, S. (2025). Epigenetic regulation and targeted therapy in gall bladder cancer. *Journal of Biochemical and Molecular Toxicology, 39*(8), e70403.
48. Subramaniam, N., et al. (2020). Young-onset oral cancer biology. *Oral Oncology, 107*, 104759.
49. Valdez, T. A., et al. (2021). Molecular mechanisms in head and neck cancer. *Cancer Treatment Reviews, 96*, 102188.
50. Wang, Y., et al. (2020). Epigenetic alterations in cancer immunity. *Nature Reviews Cancer, 20*(8), 451–465.
51. Warnakulasuriya, S. (2020). Global epidemiology of oral cancer. *Oral Oncology, 102*, 104551.
52. Yadav, S. (2022). Molecular epidemiology of OSCC. *Journal of Oral Biology and Craniofacial Research, 12*(1), 71–78.
53. Zhang, L., et al. (2022). Salivary DNA methylation markers in oral cancer. *Clinical Cancer Research, 28*(11), 2373–2384.
54. Zhang, Y., et al. (2023). Epigenetic biomarkers for early cancer detection. *Frontiers in Oncology, 13*, 1201134.
55. Zhou, W., et al. (2022). DNA methylation and cancer progression. *Nature Reviews Clinical Oncology, 19*(8), 485–499.